Deterioration Of Atomistic Mechanisms That Bind Mutated Type IV Collagens With Integrin

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Introduction

Human type IV collagen constitutes a major component of extracellular scaffolds for the assembly and mechanical stability of certain types of tissues, especially that of glomerular basement membranes. Type IV collagen is also a vital component for interacting with cells, which is crucial for cell adhesion and differentiation. Approximately 80% of Alport Syndrome cases are caused by mutations in the COL4A5 gene encoding the α5 chain of type IV collagen. The effect of Gly missense mutation within and adjacent to the predicted integrin binding sequence on collagen-integrin binding was investigated by combining atomistic molecular simulations and recombinant collagen experiments.

Experimental Characterization

Mutants		Sequences
VCL-Int	Wild type, sequence from COL1A1	GARGERGFPGERGVQGPP
9Ins	Context sequences from COL1A1	GPPGAAGVMGARGERGFPGERGVQGPP
8Ins		GPPGAA–VMGARGERGFPGERGVQGPP
9CK	Context sequences from COL4A5	GPPGAAGVMGPPGPPGFPGERGQKGDEGPP
CK	Wild type, sequence from COL4A5	GPPGAA–VMGPPGPPGFPGERGQKGDEGPP
G400E	Gly missense mutation within sequence from COL4A5 in AS carrier	GPPGAA–VMGPPGPPEFPGERGQKGDEGPP
G406V		GPPGAA–VMGPPGPPGFPGERVQKGDEGPP
G409D		GPPGAA–VMGPPGPPGFPGERGQKDDEGPP

Table 1: Sequences of recombinant collagen

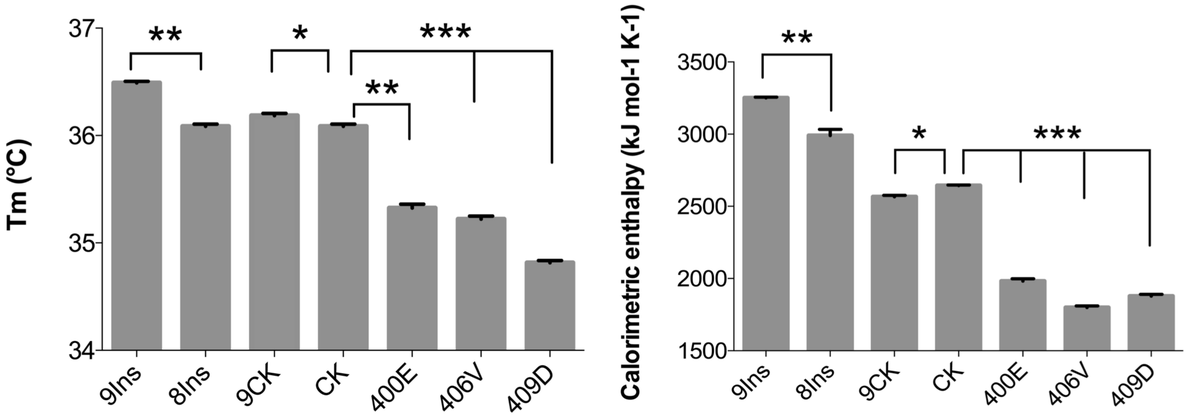

Figure 1: Mutations caused significant reduction in the melting temperatures (left) and calorimetric enthalpy (right)

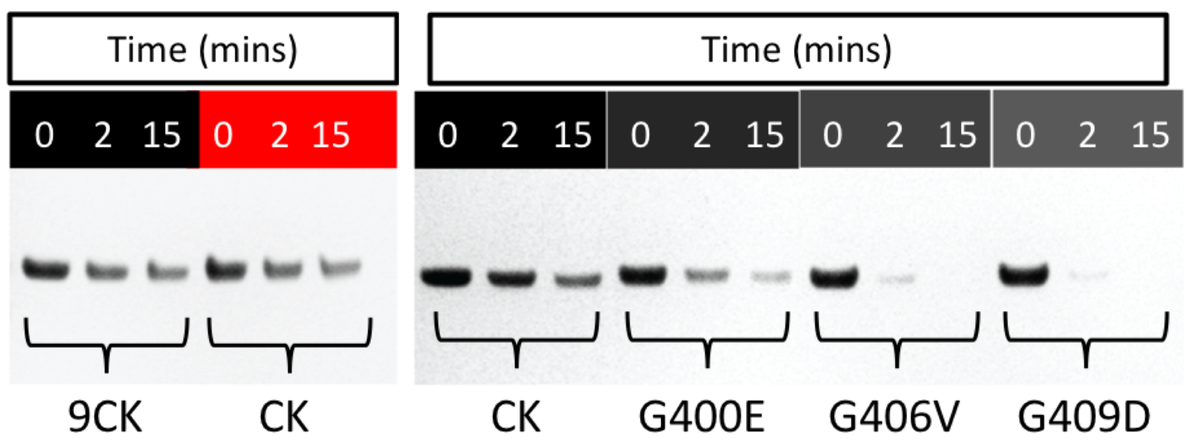

Figure 3: Reduction in trypsin resistance due to mutations

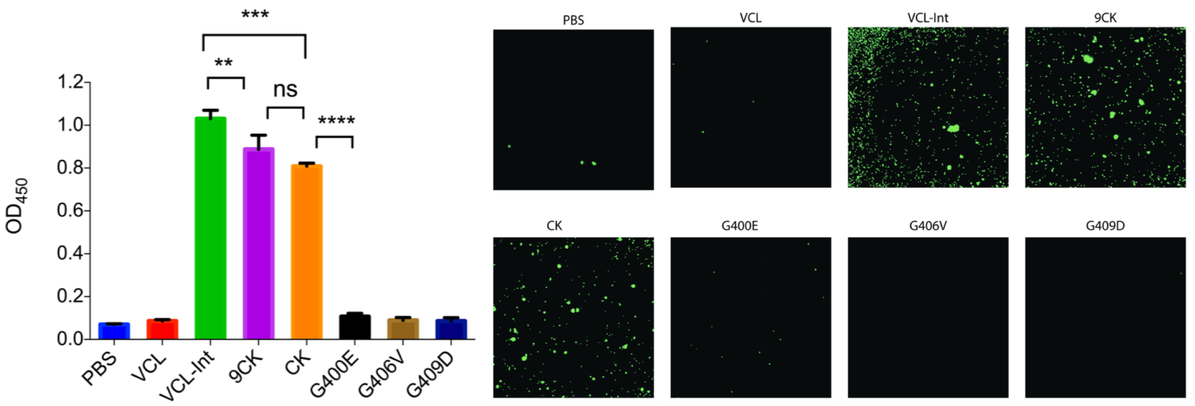

Figure 4: Reduction in integrin binding affinity and cell adhesion due to mutations

Molecular Dynamics Modeling

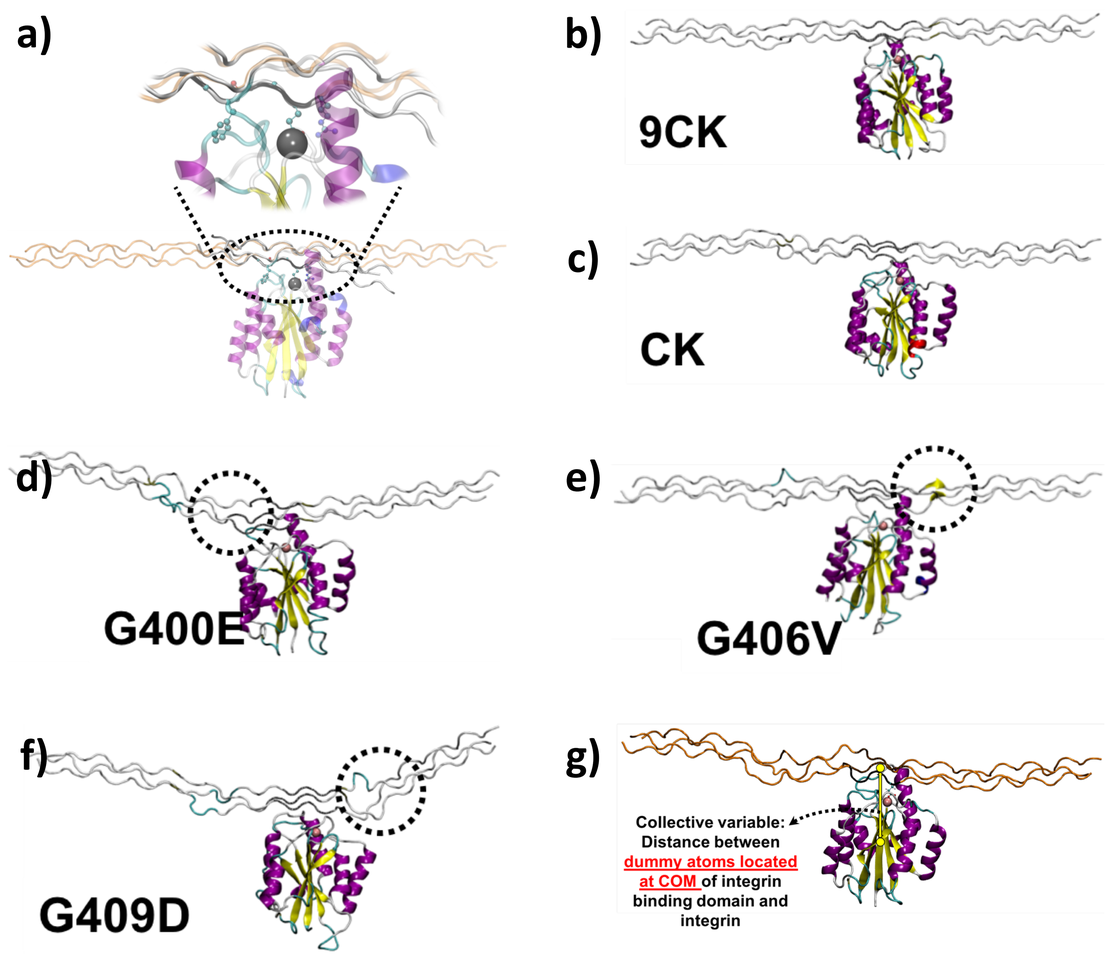

Figure 5: (a) The integrin binding domain on collagen IV is aligned with the crystal structure (PDB: 1DZI) to obtain the collagen-integrin complex. Subsequent equilibration with replica exchange with solute tempering showed that (b, c) wild-type collagen peptides were stable whereas (d-f) mutated peptides showed significant kinking or misfolding near the sites of the mutation. (g) The free energy of binding will be measured with the method of Adaptive Biasing Force for quantitative comparison with experimental data.

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Computational Clusters:

- Extreme Science and Engineering Discovery Environment (XSEDE)
- Engaging Supercomputing Center
- A*STAR Computational Resource Centre, Singapore
- National Supercomputing Center, Singapore